

From: Murphy, Joseph P Maj USMC DARPA DIRO (USA) <[REDACTED]>

Sent:

To:

Cc:

Subject:

Capt xxxxx,

Thanks for responding.

I'm reaching out to communicate some information relative to COVID that I don't believe xxxxx or your director is aware of. You probably saw earlier this week that more official documents linking NIH and EcoHealth Alliance to the Wuhan Institute of Virology were published by The Intercept. I came across additional incriminating documents and produced an analysis shortly after leaving DARPA last month. This report was routed to the DOD IG office.

I'm unsure whether the significance of what I communicated is understood by those that received the report. Decisions with regards to the vaccines do not appear to be informed by analysis of the documents. The main points being that SARS-CoV-2 matches the SARS vaccine variants the NIH-EcoHealth program was making in Wuhan; that the DOD rejected the program proposal because vaccines would be ineffective and because the spike proteins being inserted into the variants were deemed too dangerous (gain-of-function); and that the DOD now mandates vaccines that copy the spike protein previously deemed too dangerous. To me, and to those who informed my analysis, this situation meets no-go or abort criteria with regards to the vaccines until the toxicity of the spike protein can be investigated. There's also information within the documents about which drugs effectively treat the program's SARS-CoVs.

Thus why I'm reaching out. I'm trying to help aid leadership grapple with the vaccines and the mandate with as much information as is available. I wanted to push this information your way.

Several of the documents referenced in the IG report have since been downgraded.

Please reach out to me with questions.

V/R,

Major Joe Murphy USMC
Marine Program Liaison
Code 34 & 35

Office of Naval Research

Work: [REDACTED]

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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY
675 NORTH RANDOLPH STREET
ARLINGTON, VA 22203-2114

13 Aug 21

From: COMMANDANT OF THE MARINE CORPS FELLOW, DARPA
To: INSPECTOR GENERAL

Subj: SARS-CoV-2 ORIGINS INVESTIGATION WITH US GOVERNMENT PROGRAM
UNDISCLOSED DOCUMENT ANALYSIS

- Ref: (1) Executive Slide HR00118S0017 EcoHealth Alliance DEFUSE
(2) HR00118S0017-PREEMPT-FP-019-FM Summary (Selectable - Not Recommended)
(3) PREEMPT Volume 1 no ESS HR00118S0017 EcoHealth Alliance DEFUSE
(4) PREEMPT Volume 2 EHA Final HR00118S0017 EcoHealth Alliance DEFUSE
(5) SF424_2_0-V2.0 HR00118S0017 EcoHealth Alliance DEFUSE
(6) WIV Budget packet HR00118S0017 EcoHealth Alliance DEFUSE
(7) WS00094394-RR_KeyPersonExpanded_2_0-V2.0 HR00118S0017 EcoHealth Alliance DEFUSE
(8) WS00094394-RR_PersonalData_1_2-V1.2 HR00118S0017 EcoHealth Alliance DEFUSE

1. SARS-CoV-2 is an American-created recombinant bat vaccine, or its precursor virus. It was created by an EcoHealth Alliance program at the Wuhan Institute of Virology (WIV), as suggested by the reporting surrounding the lab leak hypothesis. The details of this program have been concealed since the pandemic began. These details can be found in the EcoHealth Alliance proposal response to the DARPA PREEMPT¹¹ program Broad Agency Announcement (BAA) HR00118S0017, dated March 2018¹¹ - a document not yet publicly disclosed.

The contents of the proposed program are extremely detailed. Peter Daszak lays out step-by-step what the organization intends to do by phase and by location. The primary scientists involved, their roles, and their institutions are indicated. The funding plan for the WIV work is its own document. The reasons why nonpharmaceutical interventions like masks and medical countermeasures like the mRNA vaccines do not work well can be extrapolated from the details. The reasons why the early treatment protocols work as curatives are apparent.

SARS-CoV-2's form as it emerged is likely as a precursor, deliberately virulent, humanized recombinant SARSr-CoV that was to be reverse engineered into a live attenuated SARSr-CoV bat vaccine. Its nature can be determined from analysis of its genome with the context provided by the EcoHealth Alliance proposal. Joining this analysis with US intelligence collections on Wuhan will aid this determination.

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When synthesized with the EcoHealth Alliance proposal, US collections confirm EcoHealth Alliance was performing the work proposed. The analysts produce their reports in a vacuum, absent the context the proposal provides. As a fellow at DARPA, I could see both, and can do the synthesis. For instance, WIV personnel identified in intelligence reports are named in the proposal, these people use the lexicon of the proposal in the collections, and the virus variants proposed for experimentation are identical to those gleaned by collections. Moreover, I am also privy to information obtained by congressional office investigators and by DRASTIC^{iv}, which further corroborates that the program detailed in the BAA response was conducted until it was shut down in April 2020.

The purpose of the EcoHealth program, called DEFUSE^v in the proposal, was to inoculate bats in the Yunnan, China caves where confirmed SARS-CoVs were found. Ostensibly, doing this would prevent another SARS-CoV pandemic; the bats' immune systems would be reinforced to prevent a deadly SARS-CoV from emerging. The specific language used is "inoculate bats with novel chimeric polyvalent spike proteins to enhance their adaptive immune memory against specific high-risk viruses."^{vi} Being defense-related, it makes sense that EcoHealth submitted the proposal first to the Department of Defense, before it settled with NIH/NIAID. The BAA response is dated March 2018 and was submitted by Peter Daszak, president of EcoHealth Alliance.

DARPA rejected the proposal because the work was too close to violating the gain-of-function (GoF) moratorium.^{vii} despite what Peter Daszak says in the proposal (that the work would not^{viii}). As is known, Dr. Fauci with NIAID did not reject the proposal. The work took place at the WIV and at several sites in the US, identified in detail in the proposal.^{ix}

The EcoHealth Alliance response to the PREEMPT BAA is placed along with other proposal documents in the PREEMPT folder on the DARPA Biological Technologies Office JWICS (top secret) share drive, address: Network/filer/BTO/CI Folder/PREEMPT

This folder was empty for a year. The files, completely unmarked with classification or distribution data, were placed in this folder in July 2021, which conspicuously aligns with media reporting, my probing, and Senator Paul's inquiry into NIH/NIAID gain-of-function programs. The unmarked nature combined with the timing signals that the documents were being hidden. No files at DARPA go unmarked in classification or distribution, including proprietary documents. Furthermore, PREEMPT is an unclassified program.

The files are also now held by Marine Corps Intelligence Activity (MCIA). They are identified in the reference block above.

2. SARS-CoV-2, hereafter referred to as SARSr-CoV-WIV, is a synthetic spike protein chimera engineered to attach to human ACE2 receptors and

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inserted into a recombinant bat SARSr-CoV backbone. It is likely a live vaccine not yet engineered to a more attenuated state that the program sought to create with its final version. It leaked and spread rapidly because it was aerosolized so it could efficiently infect bats in caves, but it was not ready to infect bats yet, which is why it does not appear to infect bats. The reason the disease is so confusing is because it is less a virus than it is engineered spike proteins hitch-hiking a ride on a SARSr-CoV quasispecies swarm. The closer it is to the final live attenuated vaccine form, the more likely that it has been deattenuating since initial escape in August 2019.

The utility of certain countermeasures can be extrapolated from the documents:

- The team selected for SARSr-CoV, that were most monoclonal antibody and vaccine resistant.
- It is not practical to inoculate bats directly with shots, nor can bats get respiratory infections from droplets, so the team developed an aerosol to deliver the inoculations directly into the caves. To ensure it worked well, they developed the aerosol against masked rivets.
- The proposal notes that interferon, Remdesivir, and chloroquine phosphate inhibit SARSr-CoV viral replication.

Because of its (now) known nature, the SARSr-CoV-WIV's illness is readily resolved with early treatment that inhibits the viral replication that spreads the spike proteins around the body (which induce a harmful overactive immune response as the body tries to clear the spikes from the ACE2 receptors). Many of the early treatment protocols ignored by the authorities work because they inhibit viral replication or modulate the immune response to the spike proteins, which makes sense within the context of what EcoHealth was creating. Some of these treatment protocols also inhibit the action of the engineered spike protein. For instance, Ivermectin (identified as curative in April 2020) works throughout all phases of illness because it both inhibits viral replication and modulates the immune response. Of note, chloroquine phosphate (Hydroxychloroquine, identified April 2020 as curative) is identified in the proposal as a SARSr-CoV inhibitor, as is interferon (identified May 2020 as curative).

The gene-encoded, or "mRNA," vaccines work poorly because they are synthetic replications of the already-synthetic SARSr-CoV-WIV spike proteins and possess no other epitopes. The mRNA instructs the cells to produce synthetic copies of the SARSr-CoV-WIV synthetic spike protein directly into the bloodstream, wherein they spread and produce the same ACE2 immune storm that the recombinant vaccine does. Many doctors in the country have identified that the symptoms of vaccine reactions mirror the symptoms of the disease, which corroborates with the similar synthetic nature and function of the respective spike proteins.

The vaccine recipient has no defense against the bloodstream entry, but their nose protects them from the recombinant spike protein quasispecies during "natural infection" (better termed as aerosolized inoculation).

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Furthermore, the EcoHealth proposal states that a "vaccine approach lacks sufficient epitope coverage to protect against quasispecies of coronavirus."¹⁰ Consequently, they were trying to make vaccines work by "targeted immune boosting via vaccine inoculators using chimeric polyvalent recombinant spike proteins."¹¹ The nature of using a spike protein vaccine with one epitope against a spike protein vaccine with a quasispecies may explain the unusual (and potentially detrimental) antibody response amongst the vaccinated to the new COVID variants.¹² Fundamentally, the knowledge the proposal provides signals that the risk of Antibody Dependent Enhancement (ADE) from vaccination should be evaluated with high priority, on top of the reality that single-epitope vaccines will have little effect against SARSr-CoV-WIV, as indicated in the proposal.

The potential for SARSr-CoV-WIV to deattenuate requires immediate attention. Live vaccines have been found to deattenuate in the past. If this is the case with SARSr-CoV-WIV, then the mass vaccination campaign actually performs an accelerated gain-of-function for it. Since it is designed for bats off of a human-susceptible SARS-CoV, vaccinating humans against it actually gains its function back towards a more deattenuated human-susceptible form. Improving the SARSr-CoV-WIV spike protein to gain robustness against monoclonal vaccines is one of the steps of the DEFUSE program. The mechanism to improve the SARSr-CoV-WIV spike protein (other than direct engineering) is to challenge it against animals that have spike protein-only antibodies. The attenuated virus will either die or adapt its form to neutralize the spike protein-only antibodies. The intent was to perform this task against humanized mice and then "batified" mice. Instead, it was done with the world's population.

SARSr-CoV-WIV is not meant to kill the bats, but to immunize them. This nature may explain its general harmlessness to most people, and its harmfulness to the old and comorbid, who are in general more susceptible to vaccine reactions. The asymptomatic nature is also explained by the bat vaccine-intention of its creators (a good vaccine does not generate symptoms). Such effects would be expected of an immature vaccine, or a vaccine being reverse engineered from a more virulent form into an attenuated form. The spike protein effect on ACE2 receptors exacerbates the harmfulness in accordance with age and comorbidity. The nature of SARSr-CoV-WIV's deattenuation will also indicate future virulence, though knowing its nature at last neutralizes the threat as effective treatments can be applied with confidence.

3. DRASTIC and other scientists will clean up my description of SARSr-CoV-WIV's nature and progression within the DEFUSE program. This information is sufficient for an investigative report and more than enough to correct the existing pandemic strategy. Previously, the nation did not know itself, nor the adversary in the pandemic conflict. Now it knows both. The problem can be framed appropriately and specifically against a confirmed hypothesis. Limiting disease transmission can be dropped as the implied strategic end, as it is not the actual problem,

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nor is it actually feasible. The strategy will then align early treatment protocols and prophylaxis with the known curatives as ways and means. This course of action will achieve the strategic end of clinical resolution for those that are susceptible to the adverse effects from SARS-CoV-WIV inoculation.

4. I will inevitably be asked how I figured this out and how I discovered the documents. The pandemic response became the predominant focus of my fellowship efforts. DARPA worked a number of pandemic innovations and much of its team was familiar with biodefense. I had the opportunity to "sit in the back row" per se and observe and listen-in on the government's efforts. My obligation-light fellowship also allowed me to observe and read the field. This observation grew in scope to the point that it became a series of reports, like a military scout would prepare when tasked to investigate a problem.

These reports served as iterative thinking against the problem over many months. Eventually, I arrived at a hypothesis that what leaked from the WIV could be a bat vaccine or its precursor. It was feasible that the US would try to avoid a SARS-CoV outbreak by stopping it at its source, not by halting its infections amongst people, but by halting the infections amongst the bats. Americans are creative, even if imprudent, and technologically confident enough to try it. This concept seemed to fit within the PREEMPT program construct as well, and DRASTIC had discovered that some earlier specimens within the USAID PREDICT program were obtained in Africa and sent to the WIV. Moreover, the unusual nature and pathology of the virus hinted that it could be a vaccine or be vaccine-like.

A technological challenge as difficult as inoculating bats in China would be tried at DARPA first. The massive, "Manhattan Project"-level of information suppression executed by the government and the Trusted News Initiative indicates that it would be covered-up if something bad happened. The lab-leak hypothesis and squabbling between Senator Paul and Dr. Fauci indicated that the cover up was more localized. Further, an actual cover-up would be more disciplined with its paperwork. So I presumed that unclassified files would be concealed on a higher network and found them where I expected them to be. I understood what they were and their content, pushed the files off-site, and compiled this report.

8/13/2021

X *J. Murphy*

Joseph Murphy
Major, US Marine Corps
Signed by: MURPHY.JOSEPH.PATRICK.1275023554

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¹ DARPA: Defense Advanced Research Projects Agency

¹¹ PREEMPT: Preventing Emerging Pathogenic Threats

¹² DRASTIC: Decentralized Radical Autonomous Search Team Investigating COVID-19. This collection of scientists and sleuths broke open the lab leak hypothesis into the mainstream and has picked apart Chinese and World Health Organization (WHO) reports on SARS-CoV-2's origins in Wuhan.

¹³ DEFUSE: Defusing Threat of Bat-borne Coronavirus

¹⁴ PREEMPT Volume 1 no ESS HR00118S0017 EcoHealth Alliance DEFUSE. Another description used: "We will develop recombinant chimera spike proteins from known SARSr-CoVs, and those characterized by DEFUSE, using details of SARS S protein structure and host cell binding, we will sequence, reconstruct, and characterize spike trimmers and RBDs of SARSr-CoVs, incorporate them into nanoparticles or raccoon poxvirus vectors for delivery to bats."

¹⁵ Dr. James Gimbert, DARPA Program Manager states: "team's approach does potentially involve GoF/DURC research (they aim to synthesize spike glycoproteins that may bind to human cell receptors and insert them into SARS-CoV backbones to assess capacity to cause SARS-like disease.)"

¹⁶ "We will commercially synthesize SARSr-CoV S glycoprotein genes, designed for insertion into SHC014 or HIV6 molecular clone backbones (80% and 97% S protein identity to epidemic SARS-Urbani). These are BSL-3, not select agents or subject to P3CO" (they use bat SARSr-CoV backbones which are exempt)"

¹⁷ Duke NUS Medical School, UNC, USGS National Wildlife Health Center, Palo Alto Research Center, Kunning, Singapore, and Madison, WI.

¹⁸ PREEMPT Volume 1 no ESS HR00118S0017 EcoHealth Alliance DEFUSE

¹⁹ PREEMPT Volume 1 no ESS HR00118S0017 EcoHealth Alliance DEFUSE

²⁰ "For Delta, neutralizing antibodies have a decreased affinity for spike protein, while facilitating antibodies have a "strikingly increased" affinity for spike protein." Yahi, et al. "Infection-enhancing anti-SARS-CoV-2 antibodies recognize both the original Wuhan/D614G strain and Delta variants. A potential risk for mass vaccination?" *Journal of Infection*. August 9, 2021. [https://www.journalofinfection.com/article/S0950-4453\(21\)00392-9/fulltext](https://www.journalofinfection.com/article/S0950-4453(21)00392-9/fulltext)

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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY
675 NORTH RANDOLPH STREET
ARLINGTON, VA 22203-2114

PM SUMMARY SHEET
SOURCE SELECTION SENSITIVE

Solicitation Number: HR001118S0017
Solicitation Title: PREventing EMerging Pathogenic Threats (PREEMPT)
PM Name: James Gimlett
Proposer: EcoHealth Alliance
Proposal Title: Project DEFUSE: Defusing the Threat of Bat-borne Coronaviruses
Proposal Identifier: HR001118S0017-PREEMPT-PP-019

I have reviewed the attached proposal and Evaluation Reports and find that this proposal is selectable based on the evaluation criteria included in the BAA. However, I am not recommending funding at this time based on the rationale provided below.

Funding Requested (by proposer):

Phase I	Phase II	Total
\$8,411,546	\$5,797,699	\$14,209,245

This proposal aims to identify and model spillover risk of novel, pandemic-potential SARS-related coronaviruses (SARSr-CoVs) in Asia, focusing specifically on known hotspot bat caves in China. In prior work under USAID Predict, the team identified high risk of SARSr-CoVs in specific caves in Asia. The project has a good running start since the hotspot caves already test positive, with high prevalence, for several SARSr viruses so the team won't be looking for needles in haystacks. The team will build on past surveillance work as well as some impressive work in developing geo-based risk maps of zoonotic hotspots based on past spillovers and ecological data. Two approaches are proposed to preempt zoonotic spillover through reduction of viral shedding in the bat caves: 1) Innate immune boosting to downregulate viral regulation; 2) targeted immune boosting via vaccine inoculations using chimeric polyvalent recombinant spike proteins to protect against specific high risk viruses.

Two of three reviewers marked this proposal as Selectable. Key strengths are the experienced team and the selected coronavirus hotspot caves that show high prevalence for novel bat coronaviruses. Experimental in vivo and in vitro work is logically thought out and will be used to validate molecular and evolutionary models. Proposed preemption approaches, while somewhat conventional, have the advantage of a fast timeline for validation on bat or "batenized" mouse models. Multiple vaccine delivery mechanisms are proposed, including aerosolized spray, transdermal nanoparticle application, and edible adhesive gels. However, several weaknesses to the proposal were also noted. These include a lack of detail regarding data, statistical analyses and model development and how prior work will be leveraged and extended. Proposal also lacks clear decision points to assess the deployment and validation of TA2 preemption methods in the

SUMMARY OF PROPOSED COSTS

Wuhan Institute of Virology (WIV)

DARPA-BAA-HR00111890017

	PHASE 1		PHASE 2		PROJECT TOTAL
	BASE 1 12/1/2019 Through 11/30/2020	BASE 2 12/1/2019 Through 11/30/2020	OPTION 1 12/1/2020 Through 11/30/2021	OPTION 2 12/1/2021 Through 5/31/2022	
Direct Labor - Senior and Key Personnel	37,875	37,875	37,875	22,153	136,078
Direct Labor - Other Personnel	37,027	40,524	40,824	16,987	137,682
Fringe Benefits	22,500	23,639	23,639	12,341	82,119
Total Direct Labor & Fringe Benefits	97,502	102,438	102,438	53,481	355,859
Materials and Supplies	167,651	198,167	210,887	58,507	843,113
Travel	16,735	7,282	15,823	8,027	47,571
Equipment	0	0	0	0	0
Other Direct Costs	8,200	6,200	6,200	8,200	28,800
Total Other Direct Costs	162,600	241,849	232,410	82,824	719,484
Subtotal: Direct Labor, Fringe, Overhead & Other Direct Co	260,102	314,067	334,848	136,305	1,075,345
Exclusion(s) From Base For F&A	0	0	0	0	0
Adjusted Base for F&A	260,102.25	314,067.15	334,848.25	136,305.25	1,075,342.90
F&A	28,010.00	31,409.00	33,495.00	13,631.00	107,535.00
Total Proposed Cost	319,112.25	345,486.15	368,333.25	149,936.25	1,192,877.90

10.0%

10.0%

10.0%

DIRECT LABOR BREAKDOWN										
PROJECT DEFUSE	PHASE ONE - BASE PERIOD (24 months)									
	BASE 1					BASE 2				
	Hourly Rate	# Months	# Hours	Total Salary Amount Y1	Hourly Rate	# Months	# Hours	Total Salary Amount Y2		
Personnel										
Investigator	\$25.56	3.00	528	\$13,488	\$25.56	3.00	528	\$13,488		
Dr. Peng Zhou (Senior Scientist)	\$18.28	6.00	1056	\$19,280	\$18.28	6.00	1056	\$19,280		
Dr. Ben Hu (Research Fellow)	\$10.95	3.00	528	\$5,784	\$10.95	3.00	528	\$5,784		
Associate Professor	\$13.89	6.00	1056	\$14,460	\$13.89	6.00	1056	\$14,460		
Senior Technician	\$10.95	6.00	1056	\$11,568	\$10.95	6.00	1056	\$11,568		
Technician 1	\$7.30	9.00	1584	\$11,568	\$7.30	6.00	1056	\$7,712		
Technician 2					\$7.30	6.00	1056	\$7,712		
TOTAL DIRECT LABOR				\$76,156				\$80,012		
	Rate		Base Amount	Total Fringe Y1	Rate		Base Amount	Total Fringe Y2		
	30.00%		\$76,156.13	\$22,846.84	30.00%		\$80,012.31	\$24,003.68		
FRINGE BENEFITS				\$22,846.84				\$24,003.68		
Fringe				\$99,002.97				\$104,016.00		

Total Labor	summary	e
\$358,179.69	355.859	#####

DIRECT LABOR BREAKDOWN										
PROJECT DEFUSE	PHASE TWO - OPTION PERIOD (18 months)									
	OPTION 1					OPTION 2				
	Hourly Rate	# Months	# Hours	Total Salary Amount Y3	Hourly Rate	# Months	# Hours	Total Salary Amount Y3.6		
Personnel										
Dr. Zhengli Shi (Co-Investigator)	\$25.56	3.00	528	\$13,488	\$25.56	3.00	528	\$13,488		
Dr. Peng Zhou (Senior Scientist)	\$18.28	6.00	1056	\$19,280	\$18.28	6.00	1056	\$19,280		
Dr. Ben Hu (Research Fellow)	\$10.95	3.00	528	\$5,782	\$10.95	2.00	352	\$3,954		
Associate Professor	\$13.89	6.00	1056	\$14,457	\$13.89	3.00	528	\$7,228		
Senior Technician	\$10.95	6.00	1056	\$11,563	\$10.95	3.00	528	\$5,782		
Technician 1	\$7.30	6.00	1056	\$7,709	\$7.30	3.00	528	\$3,854		
Technician 2	\$7.30	6.00	1056	\$7,709		3.00	528	\$0		
TOTAL DIRECT LABOR				\$79,997				\$39,367		
	Rate		Base Amount	Total Fringe Y3	Rate		Base Amount	Total Fringe Y3.6		
	30.00%		\$79,997.25	\$23,999.16	30.00%		\$39,367.12	\$11,807.14		
FRINGE BENEFITS				\$23,999.16				\$11,807.14		
TOTAL LABOR (Salary + Fringe)				\$103,996.41				\$51,174.28		

[illegible]

Wuhan Institute of Virology - SUMMARY COST BUILDUP BY TASK

DARPA-BAA-HR0011850012
PROJECT DEFUSE

Task #	TECHNICAL AREA / TASK	PHASE 1		PHASE 2		TASK TOTAL	
		Base 1		Base 2			
		12/1/18 - 11/30/19	12/1/19 - 11/30/20	12/1/20 - 11/30/21	12/1/21 - 5/31/22		
TA1-P1-TB-1	PCR screening of bat specimens from target bat species	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38	
TA1-P1-TB-2	Genetically sequence SARS-CoV spike proteins from PCR-positive samples	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-3	Design, produce immunoprecipitation system (LPS) assays to high- and low- jump risk SARS-CoV QSG we have characterized	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-4	Determine specificity of LPS assays by recombinant protein or attenuated virus inoculation into rabbits	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-5	Validate LPS assays using positive serum samples from protein-based LPS and viral neutralization	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-6	Test previously-collected human sera from Yunnan Province to assess SARS-CoV QSG spillover	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-7	Test targeted immune boosting in wild-caught caprine Rhinolophus spp.	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-8	Develop chimeric SARS-CoV 5' immunogens	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-9	Design and test 2nd generation chimeric 5' immunogen	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-10	Immunogens in humanized mice	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-11	Test targeted immune boosting in wild-caught caprine Rhinolophus spp.	\$ 38,388.46	\$ 38,388.46	\$ -	\$ -	\$ 76,776.92	
TA1-P1-TB-12	Identify specific sites (entry, exit points), identify 7A1, 7A2, 7A3, 7A4, 7A5, 7A6, 7A7, 7A8, 7A9, 7A10, 7A11, 7A12, 7A13, 7A14, 7A15, 7A16, 7A17, 7A18, 7A19, 7A20, 7A21, 7A22, 7A23, 7A24, 7A25, 7A26, 7A27, 7A28, 7A29, 7A30, 7A31, 7A32, 7A33, 7A34, 7A35, 7A36, 7A37, 7A38, 7A39, 7A40, 7A41, 7A42, 7A43, 7A44, 7A45, 7A46, 7A47, 7A48, 7A49, 7A50, 7A51, 7A52, 7A53, 7A54, 7A55, 7A56, 7A57, 7A58, 7A59, 7A60, 7A61, 7A62, 7A63, 7A64, 7A65, 7A66, 7A67, 7A68, 7A69, 7A70, 7A71, 7A72, 7A73, 7A74, 7A75, 7A76, 7A77, 7A78, 7A79, 7A80, 7A81, 7A82, 7A83, 7A84, 7A85, 7A86, 7A87, 7A88, 7A89, 7A90, 7A91, 7A92, 7A93, 7A94, 7A95, 7A96, 7A97, 7A98, 7A99, 7A100, 7A101, 7A102, 7A103, 7A104, 7A105, 7A106, 7A107, 7A108, 7A109, 7A110, 7A111, 7A112, 7A113, 7A114, 7A115, 7A116, 7A117, 7A118, 7A119, 7A120, 7A121, 7A122, 7A123, 7A124, 7A125, 7A126, 7A127, 7A128, 7A129, 7A130, 7A131, 7A132, 7A133, 7A134, 7A135, 7A136, 7A137, 7A138, 7A139, 7A140, 7A141, 7A142, 7A143, 7A144, 7A145, 7A146, 7A147, 7A148, 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WIV DARPA-BAA- HR001118S0017

SUMMARY COST BUILDUP BY PHASE				
	Phase I:	Phase II:	All Phases	
	24 MONTHS	16 MONTHS	42 MONTHS	
	12/1/17 - 11/30/20	12/1/20 - 05/30/22	12/1/18 - 5/30/22	
Personnel	\$ 153,601	\$ 119,939	\$ 273,740	
Fringe Benefits	\$ 48,139	\$ 35,980	\$ 82,119	
Supplies	\$ 365,828	\$ 277,285	\$ 643,113	
Travel	\$ 24,021	\$ 28,550	\$ 47,571	
Other Direct Costs	\$ 14,400	\$ 14,400	\$ 28,800	
Indirect Costs	\$ 60,419	\$ 47,116	\$ 107,535	
TOTAL	\$ 664,608	\$ 518,270	\$ 1,182,878	

SUMMARY COST BUILDUP BY YEAR					
	Year 1	Year 2	Year 3	Year 4	TOTAL PROJECT
Personnel	\$ 75,002	\$ 78,799	\$ 78,799	\$ 41,140	\$ 273,740
Fringe Benefits	\$ 22,500	\$ 23,639	\$ 23,639	\$ 12,341	\$ 82,119
Supplies	\$ 187,861	\$ 198,167	\$ 210,687	\$ 66,597	\$ 843,113
Travel	\$ 16,739	\$ 7,282	\$ 15,523	\$ 8,027	\$ 47,571
Other Direct Costs	\$ 8,200	\$ 8,200	\$ 8,200	\$ 8,200	\$ 28,800
Indirect Costs	\$ 29,010	\$ 31,408	\$ 33,485	\$ 13,631	\$ 107,535
TOTAL	\$ 319,112	\$ 345,496	\$ 368,333	\$ 149,938	\$ 1,182,878

Wuhan Institute of Virology - SUMMARY COST BUILDUP BY TASK

DARPA-SAA-HR0011185007
WIV
PROJECT DEFUSE

Task #	TECHNICAL AREA / TASK	PHASE 1		PHASE 2		TASK TOTAL
		Base 1	Base 2	Option 1	Option 2	
		12/1/18 - 11/30/19	12/1/19 - 11/30/20	12/1/20 - 11/30/21	12/1/21 - 5/31/22	
TA1-P1-T1.1	PCR screening of longitudinal specimens from target bat species	\$ 39,456.92	\$ 38,388.46	\$ -	\$ -	\$ 72,845.38
TA1-P1-T1.2	Genetically sequence SARS-CoV RNA proteins from PCR-positive samples	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.3	Design Luchuan Immunoprecipitation system (LIPS) assays to high- and low-risk SARS-CoV QSDs we have characterized	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.4	Determine specificity of LIPS assays by recombinant protein or attenuated virus inoculation into rabbits	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.5	Validate LIPS assays using positive serum samples, spike protein based LIPS and viral neutralization	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.6	Test previously collected human sera from Yunnan Province for assess SARS-CoV QSD spillover	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.7	Test targeted immune boosting in wild-caught captive Rhinolophus spp.	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.8	Develop chimeric SARS-CoV S immunogens	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.9	Design and test 2nd generation chimeric S glycoprotein immunogen in humanized mice	\$ 35,456.92	\$ 38,388.46	\$ -	\$ -	\$ 73,845.38
TA1-P1-T1.10	Test targeted immune boosting in wild-caught captive Rhinolophus spp.	\$ -	\$ -	\$ 61,388.88	\$ 24,989.38	\$ 86,378.25
TA1-P1-T1.11	Identify specific sites (entry, exit points), identify HLA epitopes, seroconversion points, fine-tune deployment plan	\$ -	\$ -	\$ 61,388.88	\$ 989.38	\$ 86,378.25
TA1-P1-T1.12	Conduct bat viral surveillance of one vaccinated cave and two control caves at our cave complex to assess baseline data for 4 months before deployment proof-of-concept experiment (IEHA Consultant Zhu, WIV)	\$ -	\$ -	\$ 61,388.88	\$ 24,989.38	\$ 86,378.25
TA1-P1-T1.13	Run deployment experiment or mock effective immune boosting molecules and delivery techniques via HLA seroconversion mechanism at one test and two control bat cave sites in Yunnan, China	\$ -	\$ -	\$ 61,388.88	\$ 24,989.38	\$ 86,378.25
TA1-P1-T1.14	Conduct bat viral surveillance of one vaccinated cave and two control caves at our cave complex to assess baseline data for 4 months after deployment proof-of-concept experiment	\$ -	\$ -	\$ 61,388.88	\$ 24,989.38	\$ 86,378.25
TA1-P1-T1.15	Assess efficacy of proof-of-concept trial.	\$ -	\$ -	\$ 61,388.88	\$ 24,989.38	\$ 1,182,877.90

Wuhan Institute of Virology DARPA-BAA-HR001118SD017	summary	9
	Total Labor	\$358,179.89
		355,850 #####

DIRECT LABOR BREAKDOWN										
PROJECT DEFUSE	PHASE ONE - BASE PERIOD (24 months)									
	BASE 1					BASE 2				
	Hourly Rate	# Months	# Hours	Total Salary Amount Y1	Hourly Rate	# Months	# Hours	Total Salary Amount Y2		
Personnel										
Investigator	\$25.58	3.00	528	\$13,488	\$25.58	3.00	528	\$13,488		
Dr. Peng Zhou (Senior Scientist)	\$18.28	6.00	1056	\$19,283	\$18.28	6.00	1056	\$19,283		
Dr. Ben Hu (Research Fellow)	\$10.95	3.00	528	\$5,784	\$10.95	3.00	528	\$5,784		
Associate Professor	\$13.69	6.00	1056	\$14,457	\$13.69	6.00	1056	\$14,457		
Senior Technician	\$10.95	6.00	1056	\$11,583	\$10.95	6.00	1056	\$11,583		
Technician 1	\$7.30	6.00	1056	\$7,709	\$7.30	6.00	1056	\$7,712		
Technician 2	\$7.30	6.00	1056	\$7,709	\$7.30	6.00	1056	\$7,712		
TOTAL DIRECT LABOR				\$76,166				\$80,012		
	Rate		Base Amount	Total Fringe Y1	Rate		Base Amount	Total Fringe Y2		
	30.00%		\$78,158.13	\$22,848.84	30.00%		\$80,012.31	\$24,003.69		
FRINGE BENEFITS (Fringe)				\$99,002.97				\$104,016.00		

DIRECT LABOR BREAKDOWN										
PROJECT DEFUSE	PHASE TWO - OPTION PERIOD (18 months)									
	OPTION 1					OPTION 2				
	Hourly Rate	# Months	# Hours	Total Salary Amount Y1	Hourly Rate	# Months	# Hours	Total Salary Amount Y2		
Personnel										
Dr. Zhengli Shi (Co-Investigator)	\$25.58	3.00	528	\$13,488	\$25.58	2.00	352	\$8,992		
Dr. Peng Zhou (Senior Scientist)	\$18.28	6.00	1056	\$19,283	\$18.28	3.00	528	\$9,841		
Dr. Ben Hu (Research Fellow)	\$10.95	3.00	528	\$5,782	\$10.95	2.00	352	\$3,854		
Associate Professor	\$13.69	6.00	1056	\$14,457	\$13.69	3.00	528	\$7,228		
Senior Technician	\$10.95	6.00	1056	\$11,583	\$10.95	3.00	528	\$5,782		
Technician 1	\$7.30	6.00	1056	\$7,709	\$7.30	3.00	528	\$3,854		
Technician 2	\$7.30	6.00	1056	\$7,709	\$7.30	3.00	528	\$3,854		
TOTAL DIRECT LABOR				\$79,997				\$58,357		
	Rate		Base Amount	Total Fringe Y1	Rate		Base Amount	Total Fringe Y2		
	30.00%		\$79,997.28	\$23,999.18	30.00%		\$58,357.12	\$17,807.14		
FRINGE BENEFITS (Fringe)				\$103,996.46				\$11,614.28		

Dear Announcer for DASHA: Please send me the following Pathologic Examination Report: **TRANSURETHRAL MPT.**

Dr. Victor Demant
President, ProHemach Alliance
460 W. 34th Street, 17th Floor
New York, NY 10001
212-380-4474

Miller Project Defunct: Defunct since removal of Bat-Bornie (banned)

Amount of the Request: Proposal: 514,209,243

Thank you for your time, and I look forward to hearing from you. If you have any questions, do not hesitate to call or email me.

YOU'LL LOVE IT:

Robert Chomura
Chief of Staff, Healthcare Alliance
460 W 34th Street, 17th Floor
New York, NY 10001
212-380-4473

Principal Investigator
Technical Project
Peter Duszak, Ph.D.
 RepHealth Alliance
 666 West 34th
 New York, NY 10018
 (212) 212-3400-4473
 (212) 212-3400-4473
 (212) 212-3400-4473

The following have been named as winners of the 1997 Award for most requested travel places and periods of performance: 1. Wilson, China (Chengdu), 1111, 105; New York, 1000, 1000; 2. London, 1000, 1000; 3. Beijing, 1000, 1000; 4. Hong Kong, 1000, 1000; 5. Shanghai, 1000, 1000; 6. Singapore, 1000, 1000; 7. Tokyo, 1000, 1000; 8. Seoul, 1000, 1000; 9. Taipei, 1000, 1000; 10. Bangkok, 1000, 1000; 11. Manila, 1000, 1000; 12. Jakarta, 1000, 1000; 13. Kuala Lumpur, 1000, 1000; 14. Singapore, 1000, 1000; 15. Hong Kong, 1000, 1000; 16. Taipei, 1000, 1000; 17. Seoul, 1000, 1000; 18. Bangkok, 1000, 1000; 19. Kuala Lumpur, 1000, 1000; 20. Singapore, 1000, 1000; 21. Hong Kong, 1000, 1000; 22. Taipei, 1000, 1000; 23. Seoul, 1000, 1000; 24. Bangkok, 1000, 1000; 25. Kuala Lumpur, 1000, 1000; 26. Singapore, 1000, 1000; 27. Hong Kong, 1000, 1000; 28. Taipei, 1000, 1000; 29. Seoul, 1000, 1000; 30. Bangkok, 1000, 1000; 31. Kuala Lumpur, 1000, 1000; 32. Singapore, 1000, 1000; 33. 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Section II

A. EXECUTIVE SUMMARY

Technical Abstract: Our goal is to define the potential for spillover of novel bat origin high-zoonotic risk SARS-related coronaviruses in Asia. In 2021 we will intensively sample bats at our field sites where we have identified high spillover risk SARS-CoV-2. We will sequence their spike proteins, reverse engineer them to conduct binding assays, and insert them into bat SARS-CoV (WNV, HCoV214) backbones (these use bat-SARS-CoV backbones, not SARS-CoV, and are exempt from dual-use and gain of function concerns) to infect humanized mice and assess capacity to cause SARS-like disease. Our modeling team will use these data to build machine-learning genotype-phenotype models of viral evolution and spillover risk. We will uniquely validate these with ecology from previously-collected human samples via LPS assays that assess which spike proteins allow spillover into people. We will build host-pathogen spatial models to predict the bat species composition of caves across Southeast Asia, parameterized with a full inventory of host-virus interubiquitous at our field sites. Then, caves in Yunnan Province, China, and a series of unique global datasets on bat host-viral relationships. By the end of Y1, we will create a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens at any site across Asia.

In 2022, we will evaluate two approaches to reduce SARS-CoV shedding in cave bats: (1) Broadscale immune boosting, in which we will inoculate bats with immune modulators to upregulate their innate immune response and downregulate viral replication; (2) Targeted immune boosting, in which we will inoculate bats with novel chimeric polyclonal recombinant spike proteins plus the immune modulator to enhance innate immunity against spillover risk viruses. We will trial inoculum delivery methods on captive bats including a novel automated aerosolization system, transdermal nanoparticle application and edible delivery. We will use stochastic simulation modeling informed by field and experimental data to characterize viral dynamics in our cave test sites, minimize timing, inoculation protocol, delivery method and efficacy of virus suppression. The most effective biologicals will be trialed in our bat cave sites in Yunnan Province, with reduction in viral shedding as proof-of-concept. **Management Approach:** Members of our collaborative group have worked together on bats and their viruses for over 15 years. The lead organization, EcoHealth Alliance, will oversee all work. EHA staff will develop models to evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for delivery of both immune boosting and immune targeting blocks. Specific work will be subcontracted to the following organizations:

- Prof. Batiz, Univ. N. Carolina, will lead targeted immune boosting work, building on his two-decade track record of reverse-engineering CoV and other virus spike proteins.
- Prof. Wang, Drexel Univ. Singapore, will lead work on broadscale immune boosting, building on his group's pioneering work on bat immunity.
- Dr. Shi, Wuhan Institute of Virology will conduct viral titration on all collected samples, binding assays and some humanized mouse work.
- Dr. Rocks, USGS National Wildlife Health Center will supervise delivery of immune modulating blocking cells, building on his vaccine delivery work in wildlife, including bat seroconversion mechanism for immune-boosting molecules.
- Dr. Unidat, PLoS One Research Center will lead development of novel delivery optimized seroconversion mechanism for immune-boosting molecules.

We are requesting \$5,109,245 total funds for the project across 2.5 project years.

Section II

C. GOALS AND IMPACT

Overview: The overarching goals of DEFUSE are to:

- Identify and model spillover risk of novel SARS-related coronaviruses (SARS-CoV-2) in Asia.
- Design and demonstrate proof-of-concept that upregulating the naturally low innate immunity of bats (broad-scale immune boosting) and targeting high-risk SARS-CoV-2 in particular (targeted immune boosting) will transiently reduce spillover risk.
- Our strategy to reduce risk of viral emergence from bats will protect the warfighter with USACOM, and will be scalable to other regions and viruses (Ebola, Hendra virus, rabies).

Innovation and Urgency: Bat harbor more emerging zoonotic viruses than any other group of mammals, are ubiquitous, abundant, and often overlooked. However, other than LPS, there is no available technology to reduce spillover risk to novel CoVs from bats, and no effective therapeutics or interventions. SARS-CoV-2 is endemic in Asia's African, and European bats' root in caves but forces in daily flight, shedding virus in their feces and urine. We have now obtained direct evidence of spillover of novel SARS-CoV-2 into people in Yunnan Province, China, close to a cave complex where we have isolated strains that produce SARS-like viruses in humanized mice but don't respond to antibody treatment or vaccination. These viruses are **Asian and distinct** due to our military and to global health security because of their **dissemination and evolution in bats and potential spillover into humans**.

EcoHealth Alliance (EHA) leads the world in predictive models of viral emergence. We will use machine-learning models of spillover hotspot, host-pathogen spatial and genotype-phenotype mapping, and unique datasets to validate and refine hotspot risk maps of viral emergence. We have shown that dampened innate immunity in bats allows them to carry otherwise lethal viruses, likely as an adaptation to the physiologic stress of flight. We will design strategies targeting molecular HCoV receptor (RUB) or Toll-like receptor (TLR) agonists, to upregulate bat immunity, and suppress viral replication, thereby significantly reducing viral shedding and spillover (broad-scale immune boosting). We will complement this by coupling agonist treatments with SARS-CoV recombinant spike proteins to boost pre-existing adaptive immune responses against specific, high-risk SARS-CoV-2 (targeted immune boosting). We will design novel delivery and automated application methods, based on our previous work on wildlife vaccines, to reduce hazard during deployment.

Technical Area 1

Our strategy begins by a complete inventory of bats and their SARS-CoV strains at our intervention test site cave complex in Yunnan, China that harbors bats with high-risk SARS-CoV. We will collect data from three types of bat communities: 1) our intervention test site and cave complex (bats on monitoring for abundance and diversity, viral prevalence and diversity, individual bat viral load and host physiological markers, genetic characterization of low- and high-risk SARS-CoV strains among bat species, roosts, and age classes; satellite telemetry and mark-recapture data on bat home range and migratory behavior; and monitoring of daily, weekly and seasonal changes in bat populations. We will use genomic data to build joint species distribution models (JSDM) to predict bat species composition of caves, and high-risk SARS-CoV diversity across S. China, South and SE Asia. These will be parameterized with BMA's database of bat host-viral relationships and estimates of zoonotic viral reservoirs for bat species. Biological inventory data on all bat caves in Southern China, the full SARS-CoV inventory from our cave test sites in Yunnan, and species distribution data for all bats. We will test and validate viral diversity predictions using data from >10,000 previously collected bat samples from 8 Asian countries under our USAID-funded PROTECT project. We will produce a prototype app for the web browser to identify the risk of bats harboring dangerous viruses at a site. This 'spatial viral spillover risk' app will be field-deployable and updated in time with surveillance data, to ground-truth and fine-tune predictions.

To characterize spillover risk of SARS-CoV equi species (QES), the Wuhan Institute of Virology team (WIV) will test bat feces, oral, and blood samples for SARS-CoV by PCR. We will collect viral load data from fresh fecal pellets. SARS-CoV spike proteins will be sequenced and recombination events identified, and isolates used to identify strains that can replicate in human cells. The Univ. N. Carolina (UNC) team will reverse-engineer spike proteins of a large sample of high- and low-risk viruses for further characterization. This will effectively freeze the QES we analyze at T=0. These QES strain viral spike glycoproteins will be synthesized, and those binding to human cell receptor ACE2 will be inserted into SARS-CoV backbones (non-DURC, non-Gof), and inoculated into humanized mice to assess capacity to cause SARS-like disease, efficacy of monoclonal therapies, the inhibitor GS-5734, or vaccines against SARS-CoV-2.

We will use these data to build machine-learning genotype-to-phenotype datasets that predict the capacity of QES strains to infect human cells based on genetic traits and experimental assays above. Using data on diversity of spike proteins, recombinant CoV, and flow of genes via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and host home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will validate model predictions of host jump risk by 1) conducting further spike

protein-based binding and cell culture experiments, and 2) identifying whether designated high-risk SARS-CoV strains have already spilled over into people near our bat cave sites. Our preliminary work shows 93% seroprevalence to bat SARS-CoV in people at this site¹⁴. We will test these previously collected human sera (>2000) for presence of antibodies to the N, Rn, and low-risk SARS-CoV-2s identified by our modeling, using Luciferase immunosuppression assay (LIPS) assays we design against the SARS-CoV-2s identified in this project¹⁵.

Technical Area 3

In TA3, we will develop scalable approaches to suppress SARS-CoV within bat reservoir species, to reduce the likelihood of virus transmission into humans. We will evaluate two approaches to defuse spillover potential: 1) Broad-scale immune boosting: we will apply immune modulators like bat interferon and TLR agonists to up-regulate bat innate immunity and suppress viral replication and shedding. 2) Targeted immune boosting: we will apply polyvalent chimeric recombinant SARS-CoV spike proteins in the presence of broad-scale immune boosting treatments to boost immune memory and suppress specific SARS-CoV. Both TA3 lines of work will run parallel beginning in a pilot phase (2020-2021) with

SPONSOR - DUE-BUSI will fund the broad-scale immune boosting work, building on its pioneering work on bat immunity¹⁶, including identifying weakened functionality of innate immunity factors like STING, a central DNA-sensor (cGAS) sensing molecule, that may allow bats to maintain an effective, but not over-response to virus¹⁷, and RNA, which is constitutively increased without stimulation¹⁸. We will trial the following, concurrently and competitively, for efficacy and scalability: I) Activating TLR/PLR pathways to induce IFN induction, e.g. poly(I:C) or 5'ppp-dRNA. A similar strategy has been demonstrated in a mouse model for SARS-CoV¹⁹. II) Universal bat interferon. Interferon has been used clinically in people, e.g., against HIV^{20,21}, and replication of SARS-CoV is sensitive to interferon²². III) Boosting bat TLR signaling using ligands. Bat TLRs is constitutively expressed but cannot be induced to a high level²³. We will use cGAS, to identify potential negative regulators and screen for compounds targeting this gene; IV) Activating dampened IFN production pathways via RNA-STRONG-dependent and RNA-TLR7 dependent pathways. Mutant bat STING retains antiviral functionality, suggesting these pathways are important in bat antiviral responses²⁴. We will directly activate the pathways downstream of STING/TLR7, to promote viral clearance; V) Vaccinating bats with SARS-CoV fragments to upregulate innate immune responses to specific CoV - a partial step towards the targeted immune boosting work below.

Prof. Benji (UNC) will lead the targeted immune boosting work. We will develop recombinant chimeric spike proteins²⁵ from known SARS-CoV, and those characterized by DEFUSE. Using details of SARS protein structure and host cell binding²⁶, we will sequence, reconstruct and characterize spike proteins and receptor binding domains of SARS-CoV, incorporate them into nanoparticles or adjuvant-like vectors for delivery to bats²⁷. In combination with immune-boosting small molecules, we will use these to boost immune memory in bats previously exposed to SARS-CoV, taking the best candidate forward for field-testing. Recombinant glycoprotein-based constructs with immunogenic blocks from across group 1B SARS-CoV should induce broad-scale adoptive immune responses that reduce immunosuppression of bat immunity in bats and unmitigated risk to people²⁸. Innate immune damping is highly conserved in all bat species tested so far. We will use the unique DUE-BUSI

Asian cave bat (Myotis species) breeding colony to conduct initial proof-of-concept tests, identified to small groups of wild-caught *Myotis* species bats at WU.

A novel delivery method for our bat vaccine technology will be developed and implemented by Dr. Jocka at the US National Wildlife Health Center (NWHC) who has previously developed unique vaccines through technology using locally acquired insectivorous bats. We will assess delivery vehicles and methods including: 1) topically applied nanoparticle; 2) orally edible bats that bat mutually groom and consume; 3) aerosolized spray triggered by timers and movement detectors at critical cave entry points. We automated probe triggered by timers and movement detectors at critical cave entry points. We

have extensive preliminary data on these techniques for wildlife, including vaccinating bats against rabies the lab¹, successful delivery, consumption and spread in wild vampire bats. We will use the NWHC captive bat colony and wild-caught bats to test delivery vehicles using the biomarker, Madenia B (which fluorescently marks hair on consumption) to assess uptake. The most optimal deployment approaches will be tested on wild bats at our test cave sites in Yunnan, using the most effective immune modulation preparations. Bat populations from experimental and control caves will be surveyed longitudinally for viral load before and after deployment trials. EXA has had unique access to these sites for ~10 years. In DEFUSE Y1, we will seek permission for experimental trials from collaborators at the Yunnan Forestry Department and Center for Disease Control, following our proven track record of rapidly obtaining IACUC and DAP/ACURA approval for animal research. We will model optimal strategies to maximize trial success for Y1, using stochastic simulation modeling of viral circulation dynamics at our sites, informed by field and experimental data. We will estimate frequency and population coverage required for our intervention, and model the time period of viral suppression, until re-colonization or evolution leads to return of a high-risk SARS-CoV.

Deliverables

- Open source models and App identifying geographical and host-specific risk of exposure for novel SARS-CoVs
- Experimentally validated genotype-phenotype models of spillover for viral strains
- Proven technology to modulate bat innate immunity to reduce viral shedding
- Tested and validated delivery mechanism for bat cave usage including vaccines in other bat host-pathogen systems (e.g. rabies, WNV)
- Proof-of-concept approach to transiently reducing viral shedding in wild bats that can be adapted for other systems including other virus.

Responsible: Dr. Jocka, Dr. T. H. Hsu, Dr. J. T. Hsu

Technical Areas

Choice of site and model host-virus system. For the past 14 years, our team has conducted CoV surveillance in bat populations across 5 China, resulting in 1,500 unique SARS-CoVs in ~10,000 samples (95% prevalence), including multiple individuals harboring the same viral strain^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165,166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,230,231,232,233,234,235,236,237,238,239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282,283,284,285,286,287,288,289,290,291,292,293,294,295,296,297,298,299,300,301,302,303,304,305,306,307,308,309,310,311,312,313,314,315,316,317,318,319,320,321,322,323,324,325,326,327,328,329,330,331,332,333,334,335,336,337,338,339,340,341,342,343,344,345,346,347,348,349,350,351,352,353,354,355,356,357,358,359,360,361,362,363,364,365,366,367,368,369,370,371,372,373,374,375,376,377,378,379,380,381,382,383,384,385,386,387,388,389,390,391,392,393,394,395,396,397,398,399,400,401,402,403,404,405,406,407,408,409,410,411,412,413,414,415,416,417,418,419,420,421,422,423,424,425,426,427,428,429,430,431,432,433,434,435,436,437,438,439,440,441,442,443,444,445,446,447,448,449,450,451,452,453,454,455,456,457,458,459,460,461,462,463,464,465,466,467,468,469,470,471,472,473,474,475,476,477,478,479,480,481,482,483,484,485,486,487,488,489,490,491,492,493,494,495,496,497,498,499,500,501,502,503,504,505,506,507,508,509,510,511,512,513,514,515,516,517,518,519,520,521,522,523,524,525,526,527,528,529,530,531,532,533,534,535,536,537,538,539,540,541,542,543,544,545,546,547,548,549,550,551,552,553,554,555,556,557,558,559,560,561,562,563,564,565,566,567,568,569,570,571,572,573,574,575,576,577,578,579,580,581,582,583,584,585,586,587,588,589,590,591,592,593,594,595,596,597,598,599,600,601,602,603,604,605,606,607,608,609,610,611,612,613,614,615,616,617,618,619,620,621,622,623,624,625,626,627,628,629,630,631,632,633,634,635,636,637,638,639,640,641,642,643,644,645,646,647,648,649,650,651,652,653,654,655,656,657,658,659,660,661,662,663,664,665,666,667,668,669,670,671,672,673,674,675,676,677,678,679,680,681,682,683,684,685,686,687,688,689,690,691,692,693,694,695,696,697,698,699,700,701,702,703,704,705,706,707,708,709,710,711,712,713,714,715,716,717,718,719,720,721,722,723,724,725,726,727,728,729,730,731,732,733,734,735,736,737,738,739,740,741,742,743,744,745,746,747,748,749,750,751,752,753,754,755,756,757,758,759,760,761,762,763,764,765,766,767,768,769,770,771,772,773,774,775,776,777,778,779,780,781,782,783,784,785,786,787,788,789,790,791,792,793,794,795,796,797,798,799,800,801,802,803,804,805,806,807,808,809,810,811,812,813,814,815,816,817,818,819,820,821,822,823,824,825,826,827,828,829,830,831,832,833,834,835,836,837,838,839,840,841,842,843,844,845,846,847,848,849,850,851,852,853,854,855,856,857,858,859,860,861,862,863,864,865,866,867,868,869,870,871,872,873,874,875,876,877,878,879,880,881,882,883,884,885,886,887,888,889,890,891,892,893,894,895,896,897,898,899,900,901,902,903,904,905,906,907,908,909,910,911,912,913,914,915,916,917,918,919,920,921,922,923,924,925,926,927,928,929,930,931,932,933,934,935,936,937,938,939,940,941,942,943,944,945,946,947,948,949,950,951,952,953,954,955,956,957,958,959,960,961,962,963,964,965,966,967,968,969,970,971,972,973,974,975,976,977,978,979,980,981,982,983,984,985,986,987,988,989,990,991,992,993,994,995,996,997,998,999,1000,1001,1002,1003,1004,1005,1006,1007,1008,1009,1010,1011,1012,1013,1014,1015,1016,1017,1018,1019,1020,1021,1022,1023,1024,1025,1026,1027,1028,1029,1030,1031,1032,1033,1034,1035,1036,1037,1038,1039,1040,1041,1042,1043,1044,1045,1046,1047,1048,1049,1050,1051,1052,1053,1054,1055,1056,1057,1058,1059,1060,1061,1062,1063,1064,1065,1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077,1078,1079,1080,1081,1082,1083,1084,1085,1086,1087,1088,1089,1090,1091,1092,1093,1094,1095,1096,1097,1098,1099,1100,1101,1102,1103,1104,1105,1106,1107,1108,1109,1110,1111,1112,1113,1114,1115,1116,1117,1118,1119,1120,1121,1122,1123,1124,1125,1126,1127,1128,1129,1130,1131,1132,1133,1134,1135,1136,1137,1138,1139,1140,1141,1142,1143,1144,1145,1146,1147,1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164,1165,1166,1167,1168,1169,1170,1171,1172,1173,1174,1175,1176,1177,1178,1179,1180,1181,1182,1183,1184,1185,1186,1187,1188,1189,1190,1191,1192,1193,1194,1195,1196,1197,1198,1199,1200,1201,1202,1203,1204,1205,1206,1207,1208,1209,1210,1211,1212,1213,1214,1215,1216,1217,1218,1219,1220,1221,1222,1223,1224,1225,1226,1227,1228,1229,1230,1231,1232,1233,1234,1235,1236,1237,1238,1239,1240,1241,1242,1243,1244,1245,1246,1247,1248,1249,1250,1251,1252,1253,1254,1255,1256,1257,1258,1259,1260,1261,1262,1263,1264,1265,1266,1267,1268,1269,1270,1271,1272,1273,1274,1275,1276,1277,1278,1279,1280,1281,1282,1283,1284,1285,1286,1287,1288,1289,1290,1291,1292,1293,1294,1295,1296,1297,1298,1299,1300,1301,1302,1303,1304,1305,1306,1307,1308,1309,1310,1311,1312,1313,1314,1315,1316,1317,1318,1319,1320,1321,1322,1323,1324,1325,1326,1327,1328,1329,1330,1331,1332,1333,1334,1335,1336,1337,1338,1339,1340,1341,1342,1343,1344,1345,1346,1347,1348,1349,1350,1351,1352,1353,1354,1355,1356,1357,1358,1359,1360,1361,1362,1363,1364,1365,1366,1367,1368,1369,1370,1371,1372,1373,1374,1375,1376,1377,1378,1379,1380,1381,1382,1383,1384,1385,1386,1387,1388,1389,1390,1391,1392,1393,1394,1395,1396,1397,1398,1399,1400,1401,1402,1403,1404,1405,1406,1407,1408,1409,1410,1411,1412,1413,1414,1415,1416,1417,1418,1419,1420,1421,1422,1423,1424,1425,1426,1427,1428,1429,1430,1431,1432,1433,1434,1435,1436,1437,1438,1439,1440,1441,1442,1443,1444,1445,1446,1447,1448,1449,1450,1451,1452,1453,1454,1455,1456,1457,1458,1459,1460,1461,1462,1463,1464,1465,1466,1467,1468,1469,1470,1471,1472,1473,1474,1475,1476,1477,1478,1479,1480,1481,1482,1483,1484,1485,1486,1487,1488,1489,1490,1491,1492,1493,1494,1495,1496,1497,1498,1499,1500,1501,1502,1503,1504,1505,1506,1507,1508,1509,1510,1511,1512,1513,1514,1515,1516,1517,1518,1519,1520,1521,1522,1523,1524,1525,1526,1527,1528,1529,1530,1531,1532,1533,1534,1535,1536,1537,1538,1539,1540,1541,1542,1543,1544,1545,1546,1547,1548,1549,1550,1551,1552,1553,1554,1555,1556,1557,1558,1559,1560,1561,1562,1563,1564,1565,1566,1567,1568,1569,1570,1571,1572,1573,1574,1575,1576,1577,1578,1579,1580,1581,1582,1583,1584,1585,1586,1587,1588,1589,1590,1591,1592,1593,1594,1595,1596,1597,1598,1599,1600,1601,1602,1603,1604,1605,1606,1607,1608,1609,1610,1611,1612,1613,1614,1615,1616,1617,1618,1619,1620,1621,1622,1623,1624,1625,1626,1627,1628,1629,1630,1631,1632,1633,1634,1635,1636,1637,1638,1639,1640,1641,1642,1643,1644,1645,1646,1647,1648,1649,1650,1651,1652,1653,1654,1655,1656,1657,1658,1659,1660,1661,1662,1663,1664,1665,1666,1667,1668,1669,1670,1671,1672,1673,1674,1675,1676,1677,1678,1679,1680,1681,1682,1683,1684,1685,1686,1687,1688,1689,1690,1691,1692,1693,1694,1695,1696,1697,1698,1699,1700,1701,1702,1703,1704,1705,1706,1707,1708,1709,1710,1711,1712,1713,1714,1715,1716,1717,1718,1719,1720,1721,1722,1723,1724,1725,1726,1727,1728,1729,1730,1731,1732,1733,1734,1735,1736,1737,1738,1739,1740,1741,1742,1743,1744,1745,1746,1747,1748,1749,1750,1751,1752,1753,1754,1755,1756,1757,1758,1759,1760,1761,1762,1763,1764,1765,1766,1767,1768,1769,1770,1771,1772,1773,1774,1775,1776,1777,1778,1779,1780,1781,1782,1783,1784,1785,1786,1787,1788,1789,1790,1791,1792,1793,1794,1795,1796,1797,1798,1799,1800,1801,1802,1803,1804,1805,1806,1807,1808,1809,1810,1811,1812,1813,1814,1815,1816,1817,1818,1819,1820,1821,1822,1823,1824,1825,1826,1827,1828,1829,1830,1831,1832,1833,1834,1835,1836,1837,1838,1839,1840,1841,1842,1843,1844,1845,1846,1847,1848,1849,1850,1851,1852,1853,1854,1855,1856,1857,1858,1859,1860,1861,1862,1863,1864,1865,1866,1867,1868,1869,1870,1871,1872,1873,1874,1875,1876,1877,1878,1879,1880,1881,1882,1883,1884,1885,1886,1887,1888,1889,1890,1891,1892,1893,1894,1895,1896,1897,1898,1899,1900,1901,1902,1903,1904,1905,1906,1907,1908,1909,1910,1911,1912,1913,1914,1915,1916,1917,1918,1919,1920,1921,1922,1923,1924,1925,1926,1927,1928,1929,1930,1931,1932,1933,1934,1935,1936,1937,1938,1939,1940,1941,1942,1943,1944,1945,1946,1947,1948,1949,1950,1951,1952,1953,1954,1955,1956,1957,1958,1959,1960,1961,1962,1963,1964,1965,1966,1967,1968,1969,1970,1971,1972,1973,1974,1975,1976,1977,1978,1979,1980,1981,1982,1983,1984,1985,1986,1987,1988,1989,1990,1991,1992,1993,1994,1995,1996,1997,1998,1999,2000,2001,2002,2003,2004,2005,2006,2007,2008,2009,2010,2011,2012,2013,2014,2015,2016,2017,2018,2019,2020,2021,2022,2023,2024,2025,2026,2027,2028,2029,2030,2031,2032,2033,2034,2035,2036,2037,2038,2039,2040,2041,2042,2043,2044,2045,2046,2047,2048,2049,2050,2051,2052,2053,2054,2055,2056,2057,2058,2059,2060,2061,2062,2063,2064,2065,2066,2067,2068,2069,2070,2071,2072,2073,2074,2075,2076,2077,2078,2079,2080,2081,2082,2083,2084,2085,2086,2087,2088,2089,2090,2091,2092,2093,2094,2095,2096,2097,2098,2099,2100,2101,2102,2103,2104,2105,2106,2107,2108,2109,2110,2111,2112,2113,2114,2115,2116,2117,2118,2119,2120,2121,2122,2123,2124,2125,2126,2127,2128,2129,2130,2131,2132,2133,2134,2135,2136,2137,2138,2139,2140,2141,2142,2143,2144,2145,2146,2147,2148,2149,2150,2151,2152,2153,2154,2155,2156,2157,2158,2159,2160,2161,2162,2163,2164,2165,2166,2167,2168,2169,2170,2171,2172,2173,2174,2175,2176,2177,2178,2179,2180,2181,2182,2183,2184,2185,2186,2187,2188,2189,2190,2191,2192,2193,2194,2195,2196,2197,2198,2199,2200,2201,2202,2203,2204,2205,2206,2207,2208,2209,2210,2211,2212,2213,2214,2215,2216,2217,2218,2219,2220,2221,2222,2223,2224,2225,2226,2227,2228,2229,2230,2231,2232,2233,2234,2235,2236,2237,2238,2239,2240,2241,2242,2243,2244,2245,2246,2247,2248,2249,2250,2251,2252,2253,2254,2255,2256,2257,2258,2259,2260,2261,2262,2263,2264,2265,2266,2267,2268,2269,2270,2271,2272,2273,2274,2275,2276,2277,2278,2279,2280,2281,2282,2283,2284,2285,2286,2287,2288,2289,2290,2291,2292,2293,2294,2295,2296,2297,2298,2299,2300,2301,2302,2303,2304,2305,2306,2307,2308,2309,2310,2311,2312,2313,2314,2315,2316,2317,2318,2319,2320,2321,2322,2323,2324,2325,2326,2327,2328,2329,2330,2331,2332,2333,2334,2335,2336,2337,2338,2339,2340,2341,2342,2343,2344,2345,2346,2347,2348,2349,2350,2351,2352,2353,2354,2355,2356,2357,2358,2359,2360,2361,2362,2363,2364,2365,2366,2367,2368,2369,2370,2371,2372,2373,2374,2375,2376,2377,2378,2379,2380,2381,2382,2383,2384,2385,2386,2387,2388,2389,2390,2391,2392,2393,2394,2395,2396,2397,2398,2399,2400,2401,2402,2403,2404,2405,2406,2407,2408,2409,2410,2411,2412,2413,2414,2415,2416,2417,2418,2419,2420,2421,2422,2423,2424,2425,2426,2427,2428,2429,2430,2431,2432,2433,2434,2435,2436,2437,2438,2439,2440,2441,2442,2443,2444,2445,2446,2447,2448,2449,2450,2451,2452,2453,2454,2455,2456,2457,2458,2459,2460,2461,2462,2463,2464,2465,2466,2467,2468,2469,2470,2471,2472,2473,2474,2475,24}

insufficient (43% prevalence) role in viral dynamics. We also measure *Myeloplasma* spp. levels using sharp trays and mice using during spawning (hot, cold, or neutral), and whole blood samples (1:2 per bait) using during a reproductive to avoid cross-contamination, and take 2 mice with a tissue punch biopsies for host DNA. In-house, host DNA receptor gene sequencing. Interactome - 3 individuals per species and one phylogenetic analysis. This will be a beneficially, and phylogenetic and physiological data (age class, sex, and physiological data) (see Table 1).

Species	n	5.45-5.60 percent
<i>Phytolophus atratus</i>	108	2.9%
<i>P. trilineatus</i>	191	0.3%

(n=3,304) of SARS-CoV in *Myotis* spp. In our study, this sample size would allow detection of 10% fluctuation in viral prevalence among sampling periods and caves. During the 2 months of capture, we will collect fecal pellets from all bats that enter the cave. To ensure that we capture a fair number with our physical bat trapping we will collect fresh fecal pellets by placing clean Ziploc polyethylene sheaths beneath roosting bats¹. *Myotis* spp. have a 7-week gestation period, spring birthing, and lactation during mating periods. Our monthly sampling strategy will collect appropriate data to parameterize stochastic simulation models, and cover two mating and two hibernation periods. We will collect data on viral prevalence and immune marker (antibody) concentration over a 1-year period to assess life-history driven changes in viral prevalence and immune marker levels. We will conduct pre- and post-hibernation sampling as follows: (1) male and female fecal pellets sampled for 4 months, and 10 male and 10 female bats per species tested every 2 weeks (post-hibernation); (2) male and female fecal pellets sampled for 4 months, and 10 male and 10 female bats per species tested every 4 months (pre-hibernation); (3) male and female fecal pellets sampled for 4 months, and 10 male and 10 female bats per species tested every 4 months (post-hibernation). Immune status changes in test and control life-bats during Phase II (P2). Immune status can be followed in individual bats due to the relatively small roost sites in these caves and our ability to identify individual bats. We will be assessing immune status using noninvasive imaging of individual bats validated during captive bat studies at Duke NUS. We will use infrared (IR) and ultraviolet (UV) light to identify individual bats. We will use ultraviolet light to identify fecal pellets and digital infrared imaging to record the number and species of bats above each cave. Polyethylene sheaths and fecal pellets will be genetically barcoded to confirm species identification. Samples will be preserved in viral transport medium, immediately frozen in liquid nitrogen, and shipped to partner laboratories with maintained cold chain and under strict biohazard safety protocols. PIV tag readers and weatherproof thermal imaging cameras mounted at each cave entrance will passively monitor temporal roost site fidelity, rates of inter-cave movement, and daily fluctuation in bat population¹⁴. ICARUS satellite transmitters (Dip) will be attached to 32 *Myotis* spp. bats from each study roost (3 bats/roost) to determine nightly foraging diurnal patterns¹⁵. Telemetry and PIV tag data will be used to

foraging dispersal pattern ()

Study caves will be surveyed using portable LIDAR technology²⁰⁰², to give a 3-D image of rock areas and data on aspects composition for targeting of intensive modulation treatment in Tab (fig. 3) Sampling results will be the adjusted based on lab and model results to

Fig. 2. Light scattering and flaring (LDA) according to characteristic curves and quantity number of flaring to be coating in section. At LDA system takes a 100° semi-circular shape of cylindrical belt. In optimize Vrst deflection.

photo converted to 3-D point cloud, C) noise points, based on laser return intensity removed, D) reconstructed bounding of gorilla counts, individual bats, Figure 1 from "

Our team has more than 50 years collective experience in safe and humane handling of dogs for biological sampling. This project will operate under appropriate IACUC/ACUHO and PPE guidelines. GMA has several ongoing ACO-supported projects, has obtained ACO approval for animal research from the DoD, and currently maintains IACUC protocols through Tufts University for lab work in a different facility, which we will use for DEFUSE IACUC. IACUCs are ready to proceed for lab work at Tufts/USC. LINC, NVMHC, and WNY will be modified for DEFUSE.

predictive models of high-risk sites and hot spatial across Asia. We will build models that predict bat and viral diversity and spillover risk across Asia to enable wringfitters and planners to assess risk and necessity for intervention deployment at ITAs. We will combine regional-scale and local species distribution models (SDMs), machine learning host-virus association models, and parametric viral richness estimators to respectively predict the composition of bat communities in caves across Asia, host range for key viral clades, and a set-of-very unimpaired viral diversity. We will use a stochastic forward neural network to implement SDMs that are effective at multiple scales with incomplete observations (as occur for bats and their viruses). We will account for bat species occurrence driven by environment or availability⁴¹. We will link our SDM to biological inventory data on over 200 caves in the region⁴², to physiologically relevant bioclimatic variables (BIOTCLIM⁴³), open source topographic data, and proxies for human disturbance such as road density and habitat heterogeneity. As in previous work⁴⁴, we will use these models with regional-scale environmental variables (land use, distance to roads, etc.) and cave-specific variables (cave length, availability of roosting areas, entrance orientation, cave complexity, etc.). We will validate them using independent bat occurrence data, and observations⁴⁵. We will use this unique database of all known bat-virus associations to assess the geographic distribution of bat CoV diversity and host range⁴⁶ (Fig. 4). We will use these data to build machine learning models and machine-learning algorithms (BNT,

random forest)¹⁸ with non-parametric estimators to predict SAREs. Cov. diversity in the US of each species¹⁹ and annual discovery rates in real (limb through sampling (Fig. 5)).

Fig. 3. Replicative rates of avian influenza viruses in China and SE Asia. Yellow bars represent the replicative rates of the H5N1 virus strains, based on an unknown minimal host range threshold. Our previous test curve is also indicated (see text). Fig. 3. *H5N1* virulence against fish with 95% confidence intervals (dashed line with 95% confidence intervals) based on the DICT sampling data could vary for four different reasons.

To extend the geographic scope of previous models, we will include data from >1000 viral sequences (Covs and others) from >10,000 individuals in samples in 6 Asian countries (NIAID-USAP H1001-CR-04), for species composition and viral presence predictions, we will include data against a 20% validation subset of data and field data.

provides a map for the waylifter. Drawing on experience building applications for data integration and processing, the book is a must read for all data professionals.

weightier that identifies probability of dangerousness, and is rolling over from bats as a

We will use outputs from our spatial risk modeling, observed and predicted host-viral

associations, open-source epidemiological and pathogen ontologies, and app-directed crowd-sourced

education data to ground-truth and fine-tune its predictive capacity. This app will be

updated in Y2 and Y3 to incorporate additional risk data from host-virus binding assays and

SARS-CoV surveys. We will use RNA's risk-ranking algorithms (1) to display

critical areas of high risk based on geolocation, temporal, location of transmission, host and

pathogen characteristics. The app will collect user GPS location data and predict bat species

distribution and community composition estimates from our ISDBs. These will be refined with

real-time surveillance data collected without the need to enter cave lists using mobile phone-

enabled high-frequency microphones for detection¹⁴, validated and trained with references

acoustic calls using convolutional neural networks¹⁵. Identified bat species will be automatically

linked with viral diversity data from RNA's host/pathogen database and SARS-CoV data from

DEFUSE to cluster high-risk pathogen taxa, displayed as pathogen-centric, bat-centric, or mega-

centric views, with proactive alerts when critical information is received.

This

technology will improve overall situational awareness of visitors and reveal infectious agents

found in bats, allowing OGB personnel to quickly identify areas high-potential risk sites and

rapidly deploy resources to respond to and mitigate their impact on ecosystems when necessary.

SARS-CoV detection, sequencing, and recovery. We will screen samples for SARS-CoV

nucleic acid using our pan-CoV consensus one-step semi-nested RT-PCR assay targeting a 448-

nt fragment in the RNA-dependent RNA polymerase gene (coding) of all known α - and β -CoV's

and qPCR assays for known SARS-CoV-2/229E. PCR products will be gel purified, sequenced

and specific assays for known SARS-CoV-positive samples to determine viral load. Full-length

genomes or 5' genes of all SARS-CoV will be RT-to-biotin-bridged sequenced followed by genome

walking^{16,17} or 5' genes of all SARS-CoV will be RT-to-biotin-bridged human ACE2 by Block-it[®] virus

walking^{18,19}. We will analyze the 5' gene for its ability to bind human ACE2 by Block-it[®] virus

entry assay. *Synthesis of Chimeraic Novel SARS-CoV-2*. We will commercially synthesize SARS-

CoV-2 glycoprotein genes, designed for insertion into 3'UTR of pCAGGS. These are 85-93 nt

backbones (88% and 97% S-protein identity to epidemic SARS-like) which are exempt) and we

select agents or subject to P3CQ (they use bat SARS-CoV backbones which are exempt) and we

pathogenic to ACE2 transgenic mice. Different backbone sequences increase recovery of viable

viral identification of barriers for RNA recombination-mediated gene transfer between

human, bat or chimeric ACE2 receptors to support cultivation of viruses with a weaker RBD-human

ACE2 interface. *Discovery of Full Length SARS-CoV-2*. We will compile sequencing/RNAseq data

from a panel of closely related strains (~5% nucleotide variation) and compare full length

genomes, scanning for unique SNPs representing sequencing errors^{24,25}. Consensus candidates

genomes-length RNA and are cryopreserved to rescue recombinant viruses^{26,27}.

Predicting strain-specific SARS-CoV spillover risk. We will characterize OS at our test cases sites with state-of-the-art sensitive pathogen surveillance networks, models. This will enable us to predict the jump probability of future OS that emerge with unique genetic recombinations. Our model will be parametrized with experimental data from a series of assays on the 3 genes of bat SARS-CoVs [Fig. 6], along with experimental and modelling work flowing together in iterative steps. Our prior data will act as baseline to parameterize spillover risk modeling analysis. This will be supplemented by characterization of isolated viruses under DEBUT or PIVET, approximately 15-20 bat SARS-CoV strains per country/year (at UNC, WNV, and XBD bat SARS-CoV strains acquired in our prior work and not yet examined for spillover potential). All experiments will be performed in triplicate and data fed into models in real time.

Experimental assay of SARS-CoV Q1S jump potential [Fig. 6, right]. *Pre-exposure via structural protein modeling:* Viral entry mechanism identification; *Infectiousness assays:* Viral entry in the major species restriction preventing spillover of SARS-CoVs⁹⁸. To select QS for further characterization we will first use structural modeling of SARS-CoV's protein binding to ACE2 receptors⁹⁹. Mutations in the RBD^{100,101,102}, and host protease proteolytic processing of the S^{103,104,105,106} may regulate SARS-CoV cell entry and cross-species infectivity. Mimicking in the RBD-ACE2 molecules or protease processing will prevent cell entry mechanisms will be diversified. Single amino acid variations phenotypes and we will evaluate the impact of low abundance in the ABD using Abxq to identify low abundant relevant to ACE2 binding. We will conduct *in vitro* pseudovirus techniques¹⁰⁷ and live virus binding assays [all WNV to preserve dissemination of viral cultures] for isolated strains. Initial data inputs will be used to guide strain selection for further receptor usage across species [WNV]. Growth in primary mammalian infectious mononuclear antibody cell recognition would indicate positive results.

Bioinformatics analyses: Should those isolates prove highly resistant to our MAb gene neutralization against a limited number of human SARS-CoV outbreak. Chimera strains that encode novel S genes will identify SARS-CoV strains for recovery full genome level sequencing of all animals lost. Secondary function SARS-CoV clinical biosynthesis loss.

feeding 2,4-Di-Cy OD have potential
harm and safety 2,4-Di-Cy OD.



p.i., and sacrificed at day 2 or 6 p.i. for virologic analysis. Histopathology and immunohistochemistry of the lung and for 22-mer retroviral genome (unt (QD) and bronchioalveolar lavage (BAL) with *Anti-NSP3* genome QD. We will analyze results from climatic viruses by re-characterizing full-length genome versions, testing whether full-length genome sequences affects full length SARS-CoV replication potential. Qs for full-genome characterization will be selected to reflect strain differences in virulence, receptor usage, growth in human cells and pathogenesis. We will test growth in primary plac cultures and in H293T transgenic mouse cells and antibiotic resowing -3.5 full length genome viruses.

Zinc-finger Synthetic Modifications. We will synthesize QS with novel combinations of mutations in Zinc-finger domains. We will analyze the effects of these mutations on the ability of the QS to determine the effects of specific perturbations and the jump potential of future and unknown recombinants. **MSO delectable.** Small deletions in the SARS-CoV RdRp alter risk of human infection. We will analyze the functional consequences of these RD deletions on SARS-CoV RdRp activity and the ability of the RdRp to replicate and transcribe the viral genome. **CoV RdRp receptor usage.** Growth in HAE cultures and in vivo pathogenesis. First, we will analyze the effects of mutations in the SARS-CoV RdRp on its ability to bind to and enter HAE cells. We will then analyze the effects of mutations in the SARS-CoV RdRp on its ability to replicate and transcribe the viral genome in HAE cells. **CoV RdRp receptor usage.** Growth in HAE cultures and in vivo pathogenesis. First, we will analyze the effects of mutations in the SARS-CoV RdRp on its ability to bind to and enter HAE cells. We will then analyze the effects of mutations in the SARS-CoV RdRp on its ability to replicate and transcribe the viral genome in HAE cells.

diates these regions, sequentially and in comparison, in 3H and 3S. Our results anticipate that the production of dilations will prevent virus growth in Yersinia and that, in parallel, we will evaluate whether RBD deletion again restores the ability of low risk strains to use human ACE2 and grow in human cells. *52* *Proteoglycans and Glycoproteins* After receptor binding, a variety of cell surface and cytosolic processes cleave the SARS-CoV-5 glycoprotein causing massive changes in its structure *53* and activating Rulon-mediated

human infection. We will analyze the functional consequences of these RBD deletions on SARS-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and in combination, in SHCov2 and SARS-CoV Urban1, anticipating that the introduction of deletions will prevent virus growth in macrophages and HAE cultures. In parallel, we will evaluate whether RBD deletions restore the ability of low risk strains to use human ACE2, and grow in human cells. *52 Proteolytic cleavage and glycosylation sites:* After receptor binding, a variety of cell surface and intracellular proteases cleave the SARS-CoV S glycoprotein causing massive changes in S structure⁷³ and activating fusion-mediated entry⁷⁴. We will analyze all SARS-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites⁷⁵. SARS-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will alter several of these sites based on pseudotyped particle studies and evaluate the impact of select SARS-CoV S changes on virus replication and pathogenesis. We will also reveal the sequence data for low abundant high risk SARS-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the respective high abundant, low risk parental strain. *Altered glycosylation sites:* Some glycosylation events regulate SARS-CoV particle binding DC-SIGN⁷⁶, alternative receptors for SARS-CoV entry into macrophages or monocytes⁷⁷. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and racoon dogs⁷⁸. While the sites are absent from civet and racoon dog strains and clade 2 SARS-CoV, they are present in WNV1, WNV46 and SHCov34, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 disrupting residues of SARS-CoV and SHCov34 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN, and in human monocytes and macrophages anticipating reduced virus growth efficiency. We will introduce the clade 1 mutations that result in N-linked glycosylation in rs4337 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ectopically expressing DC-SIGN expression⁷⁹. *In vivo:* We will evaluate pathogenesis in transgenic hACE2 mice, low abundance micro-varieties. We will structurally model and identify highly variable residues changes in the SARS-CoV S RBD, use commercial gene blocks to introduce these changes singly and in combination into the S glycoprotein gene of the low risk, parental strain and test ACE2 receptor usage, growth in HAE, and *in vivo* pathogenesis.



Fig. 7: A triangulated directed graph of a bipartite network model representing the causal relationships between input, node, model and output, and failure.

Network machine-learning to predict spillover potential of high-risk SARS-CoV strains. We will use experimental data from above to build genotype-phenotype models of bat SARS-CoV spillover potential. We will use Bayesian Networks Model (BNM), via MCMC methods¹⁶ to predict spillover risk based on bat SARS-CoV genotype data (presence of deletions in RBD, glycoprotein binding and glycosylation sites etc.) and the ecological traits of hosts - integrating data on multiple, interacting processes and CoV spillover potential to generate overall spillover probabilities. The Bayesian approach will allow us to update our models iteratively as new data is acquired, and use interim model predictions to guide which experiments to prioritize to maximize predictive ability¹⁷. We will control for experimental conditions (assays on live viral isolates, full-genome or synthetic chimera viruses, and the molecular backbone of the latter). Traits will be tested as inputs to BNMs' causal graph, to predict latent variables representing interconnected processes that contribute to SARS-CoV infection in new hosts: receptor binding, cell entry, intracellular system hijacking, and intracellular growth, all measured by our assays¹⁸. These, in turn, will act as predictors for the ultimate outcomes of host pathogenesis and host jumping potential (Fig. 1). We will use published work on these genetic traits to put informative priors on strength and direction of interactions in the causal graph. We will use prior-knowledge model simulations to select target sequences from our sampling for characterization and genome-sequencing, to collect data that maximally enhances the predictive power of our models and update these simulations iteratively throughout the experimental phase to continually guide CoV selection. We will use regularizing priors to reduce overfitting and select the most predictive variables in the final model.

Model validation using SARS-CoV serology from previously-collected human samples and surveillance data. Active spillover of SARS-CoV into study regions enables us to measure actual spillover risk to validate our models of CoV jump potential. We will gather data on viral CoV antibodies found in the host human population using virus assays on 32,000 previously-collected human samples, Darsak PI, from people living close to our test case sites in Yunnan province, a sub-sample of which shows a 2.7% seroprevalence to bat SARS-CoV¹. The BBS for

this work is current and covers proposed DEFUSE funding. We will assess UPS assays targeting high- and low-potential risk SARS-CoV-2, as done previously for SARS-CoV-2 virus and novel SARS-CoV-2. We will: 1) infect different high- and low-risk SARS-CoV-2 N gene into PERV-1 vector (UPS vector), firstpassing in gene similarity to determine their potential cross-reactivity in a UPS assay; 2) determine UPS assay specificity by producing polyclonal sera via infection of recombinant protein or attenuated virus into rabbits; 3) validate UPS assays by incubating antigen with their respective positive serum samples and the antigen antibody complex eluted using protein A/G beads; 4) validate UPS positive results by spike protein based UPS and viral neutralization assay. As a confirmatory test, the positive samples from UPS will be validated by virus neutralization assay. We will use these UPS assays to test serum samples for presence of antibodies to high- and low-risk SARS-CoV-2. We will develop predictions of jump potential and extend the ability to predict actual spillover probability by modeling to human contact rates with bats. We will use ecological data on bat hosts and human behavioral survey data collected previously from these individuals to estimate wildlife contact in predicting exposure measured by ser UPS assays.

Evolutionary modeling and simulation to predict potential spillover. Our Bayesian network modeling will generate predictions of the spillover risk of Qs sequences we identify. To examine risk associated with the total viral population, we will model and simulate evolutionary processes to identify likely viral Qs that our sampling has not captured, and viral Qs likely to arise in the future ("Qs"). We will use a large dataset of 5 protein sequences and full-length genomes generated from prior work and DEFUSE fieldwork to estimate SARS-CoV-2 substitution rates and its genome-wide variation using coalescent and molecular clock models within a Bayesian BEAST framework. We will estimate SARS-CoV-2 recombination rates at the cave population level using these data and Bayesian inference. We will apply BEAST, similar to prior work, and bootstrap to identify recombination breakpoints and hotspots within the SARS-CoV-2 genome as done previously, now extended to the full genome. Using these estimates we will simulate the evolution of the SARS-CoV-2 genome using a forward-time approach implemented in simulations that model specific RNA virus functions (e.g., VIRAPOPS). We will predict the rate at which new combinations of genetic traits can spread in viral populations and compare recombination rates among caves and bat communities. Our forward-simulated results will provide a pool of likely unknown and future Qs spacers. Using these and our SEM model for spillover risk, we will predict the Qs, most likely to arise and have spillover and pathogenic potential. We will use evolutionary simulation results to iteratively improve our Bayesian network model. The number of genetic traits with potential for prediction of pathogenicity is large, so we will perform variable reduction using tree-based clustering, retaining highly co-occurring traits as latent clusters for prediction. We will generate these clusters from all SARS-CoV-2 sequences from DEFUSE fieldwork and prior work. As trait clusters may be modified through recombination, we will use our forward-evolutionary modeling to predict how well trait clusters will be conserved, retaining only those unlikely to arise in unknown or Qs genomes. This will enable a trade-off between increased predictive power based on current samples and generalizability to future strains that have not yet evolved.

Technical Annex 2

Immune modulation approach to reducing bat SARS-CoV-2 spillover risk. Our work shows that the following unique immunological features of bats may explain their capacity to harbor high viral loads with minimal clinical signs: a) bats maintain constitutively high expression of IFN α , which may respond to and restrict viral infection¹³; b) several interferon activation pathways are downregulated, e.g. STING (a central cytosolic DNA-sensor molecule to induce interferon)¹⁴; depleted and TLR7 dependent pathways¹⁵; c) the NLRP3 dependent inflammasome pathway is dampened, and key inflammasome response genes like AIM2 are not present in bats^{16,17}. These traits may be due to bat immune-sensing pathway adaptation as a fitness cost of flight¹⁸. We hypothesize that bat virus replication will likely be restricted quickly by constitutively expressed IFNs in bats, resulting in lower EIT cell stimulation due to lower viral stimuli. Second, dampened interferon and inflammasome responses will result in lower cytokine responses that are required to trigger TLR7 cell dependent adaptive immunity (e.g. antibody response), ultimately resulting in suppression of viral replication and shedding. We and others have demonstrated proof-of-concept of this phenomenon: Experimental Marburg virus infection of Egyptian fruit bats, a natural reservoir host, resulted in widespread tissue distribution with low viral load, brief viremia, low seroconversion and slow antibody titer that waned quickly, suggesting no long-term protection is established^{19,20}. Poor neutralizing antibody responses occur after experimental infection of bats with Tacaribe virus²¹, and in our studies of experimental infection of bats with SARS-CoV (Wang, unpubl.). We also successfully showed that bat interferon can inhibit bat SARS-CoV-2. We hypothesize that use of immune modulators that upregulate the naturally low innate immunity of bats to their viruses will transiently suppress viral replication and shedding, reducing the host immune risk. We further hypothesize that because rhinovirus bats are long-lived (20+ yrs in the wild), most bats in a population will have been exposed to a range of SARS-CoV Qs at our sites. Specifically, transient suppression of their adaptive immunity (immune memory) to high-risk viral strains may lead to heightened clearance of high-risk strains. We will evaluate two immune modulation approaches to reduce spillover of SARS-CoV-2 from bats to humans: 1) Broad-spectrum immune boosting strategies (Wang, Duke-Muski) we will apply immune modulators the TLR-agonists, secret molecule 2a to receptor (TLR) agonists or bat interferon in the bats, to upregulate their innate immunity and suppress viral replication and shedding. 2) Targeted immune boosting fields. When the broad-spectrum immune boosting approach will be applied in the presence of chronic immunogens to activate immune memory in adult bats and boost clearance of high-risk SARS-CoV-2. We will use novel chimeric polyvalent recombinant proteins in microcapsules encapsulated gels for oral delivery and/or virus adjuvanted immune boosting strategies where chimeric recombinant SARS-CoV-2 are adjuvanted by microcapsules. Both lines of work will begin in Year 1 and run parallel, be assessed comparatively for efficiency, cost, and safety, and successful candidates from captive animal trials will be used in the bat trials at our test cave in Yunnan. The finding of low innate immunity across bats suggest that immune boosting could be broadly applicable to bat reservoirs and viral spillers.

